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Fruit tree genetics at a turning point: the almond example

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Abstract The large size and the long generation time of fruit trees generally reduce the possibilities of obtaining genetic information on the transmission and heritability of useful agronomic traits in these species. However, from breeding work carried out with fruit trees, an important amount of data is now available, although large differences are apparent among the different species. There is not much information known about almond compared to what is available on other *Prunus* fruit species, but more data have been accumulated on it than on most of the other nut trees, thus making almond special among all the temperate fruit and nut species. Only five qualitative traits have been described in almond, with an additional two also possibly qualitative. Heritabilities have been estimated for an important number of quantitative traits, mainly phenological times and fruit characters. Important information is available on molecular markers, including enzymes, RFLPs, RAPDs and other recently developed markers. Linkages, however, have only been established among molecular markers, allowing accurate genetic maps to be built but not yet enabling agronomical characters to be located in these maps, probably because the latter have not been sufficiently studied. The effectiveness of the application of genetic maps in plant breeding will depend on the accuracy of the study of different agronomic traits and their expression, implying more field work and recognition of this work. Ultimately, any new fruit cultivar has to be grown in the field and has to allow the grower to make a profit.

Key words Fruit trees · Genetics · Almond · *Prunus amygdalus* · Breeding

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Introduction

Fruit trees are generally large-sized and long-lived species showing a more or less pronounced juvenile period. These characteristics normally imply the requirement of large extensions to grow the seedlings and frequently reduce the number of individuals which can be handled. Also reduced is the size of the progenies that can be obtained from crosses either in the field, subject to weather contingencies, or in protected environments, subject to space limitations and often also to conditions that reduce fruit set (Socias i Company and Felipe 1992a). The long generation time also limits the possibilities of studying different generations and have fostered different approaches to shorten juvenility (Sherman and Lyrene 1983). The occurrence of self-incompatibility in many fruit species also makes it impossible to obtain pure lines. All these conditions result in a paucity of genetic studies on the structure and heritability of different traits in these species. Consequently, our genetical knowledge of fruit species is extremely reduced as compared to other crop plants, mainly cereals and vegetables, where it has been possible to construct genetic maps using only the possibilities available with classical genetics.

Although these limitations affect all fruit species, sharp differences are found among them with respect to the genetical information available for each one. This difference is due to the different conditions relating to the economical importance of each species or to the different approaches which make the genetical approach easier in some species than in others. Thus, apple (*Malus* × *domestica* Borkh.) has attracted great deal of attention from all points of view because of its economical importance since it is grown in a very large area all around the world. This attention has resulted in a vast genetic knowledge of this species (Brown 1992), in the important breeding efforts devoted to its improvement (Janick et al. 1996) and in the

multidisciplinary approaches taken to solve its growing problems. As a consequence, apple is the horticultural species with the largest number of scientific publications (Bhat 1990). Peach [*Prunus persica* (L.) Batsch] is probably the fruit species best known from the genetical point of view (Scorza and Sherman 1996), not only due to its economical importance, but also to its short juvenility and its self-compatibility which allow successive generations and self-pollinated progenies to be obtained very quickly (Monet 1989). Other very important fruit species, such as *Citrus*, are not so well known genetically probably because of the seedlessness found among many representatives of these species and the frequent production of apomictic seeds (Soost and Roose 1996).

Almond (*Prunus amygdalus* Batsch) occupies a very peculiar place among fruit trees. Although it belongs to the genus *Prunus*, which comprises all the stone fruit species, it is generally placed among the nuts, which even belong to different botanical families: walnut (*Juglans regia* L.) and pecan [*Carya illinoinensis* (Wagenh.) K. Koch] to Juglandaceae, hazelnut (*Corylus avellana* L.) to Betulaceae, pistachio (*Pistacia vera* L.) to Anacardiaceae… So, when attempting a genetical approach to almond, it is more reasonable to consider this species among the stone fruits, even if in this context our knowledge is very scarce, because almond has been much less studied than the other rosaceous fruits, which, besides the rest of the stone fruits, include the pome fruits. However, when considered as a nut, almond would be rated as a well studied species, as the scientific approaches to most of the other nut species are somewhat limited.

This peculiar place of almond makes it an interesting species to approach in order to ascertain the genetical knowledge we possess on it and how this genetical knowledge could be applied in the breeding process. My objective was to review the qualitative and quantitative traits described so far in almond, the molecular markers being currently developed and the linkage between them, as only this linkage will lead to the utilization of molecular markers in almond breeding. I also discuss the possibilities and usefulness of this utilization.

Almond origin

Almond was probably domesticated during the third millennium BC (Spiegel-Roy 1986). It has been suggested that this domestication could have taken place in Central Asia (Kovalyov and Kostina 1935) where wild almond trees can still be found (Popov et al. 1929). Many wild species that are related to almond and that intercross freely with it have been also described in this region (Browicz and Zohary 1996; Denisov 1988; Grasselly 1976). Among these species, probably *Prunus fenzliana* Fritsch., *P. bucharica* (Korsh.) Fedtsch., *P. kuramica* (Korsh.) Kitam. and *P. triloba* Lindl. have been involved in various hybridizations, giving rise to the current almond cultivars (Grasselly and Crossa-Raynaud 1980; Kester et al. 1990). Furthermore, as almond cultivation moved towards the Mediterranean, new hybridizations might have occurred, especially with the wild Mediterranean species *P. webbii* (Spach) Vierh. (Godini 1979; Socias i Company 1990), resulting in some of the almond populations found along the northern shore of the Mediterranean sea from Greece and the Balkans to Spain and Portugal.

The genetical closeness of almond and peach has led Watkins (1979) to suggest that both originated from the same primitive species but evolved separately following the mountain upheavels of the Central Asian massif. Thus peach evolved in the East, spread over several regions of China, in a more humid climate and at lower elevations, whereas almond evolved in the West, in arid steppes, deserts and mountainous areas, under severe conditions that possibly led to its evolution toward self-incompatibility. Selection for domestication would have been for sweet kernel and larger nut size among these wild populations, which were propagated by seed, the usual way of propagation still common in many regions of the world, mainly in the countries of Central Asia and the Middle East (Kester et al. 1990).

Almond was presumably introduced into the Mediterranean area through seeds carried by caravans crossing the Central Asian steppes on their way from China to the West. This manner of dispersal, has also been suggested for other fruit trees (Juniper 1996) and would work in both directions. Gustafson et al. (1988) reported that the primary sources of almond at Kashgar, Xinjiang (China) were old seedling trees which had originated from Central Asia across the Tian Shan mountains. Kashgar is on the old Silk Route connecting China and the West.

Based mainly on archaeological remains, Zohary and Hopf (1993) have put forward another hypothesis on almond origin, suggesting that it was taken into cultivation in the eastern part of the Mediterranean basin, more or less at the same time as the olive, grapevine and date palm. However, the wild populations and species found in this region are genetically more distant from the cultivated almond than the wild populations and species of Central Asia, throwing some doubts on this hypothesis, although the introduction of almond in the eastern Mediterranean area could has been as early as the second millennium BC because clear almond remains have been found in the tomb of Tutankhamon (Zohary and Hopf 1993). Almond cultivation had to have existed in Greece long before the creation of the Greek myths to explain its incorporation into them (Graves 1955); also, there is evidence of almond trade in the western Mediterranean in the fourth century BC (Cerdá Juan 1973).

Traditional almond culture primarily utilized open-pollinated seedlings (Grasselly 1972; Rikhter 1972).

This fact, together with self-incompatibility, has created a very high heterozygosity in this species, which is one of the most polymorphic fruit species (Kester et al. 1990; Socias i Company and Felipe 1992b). This large variation has been an effective genetic pool for breeding but at the same time has probably retarded its study.

Qualitative traits

In contrast to a closely related species such as peach, for which many qualitative traits have been described (Monet et al. 1996), only a few traits have been described in almond, probably because not much attention has been paid to the study of different almond progenies and also because these progenies have been obtained from parents in which these traits can not be found. So far, only five qualitative traits have been described: kernel taste, shell hardness, self-incompatibility alleles, self-compatibility, and blooming time. There are probably other qualitative traits, but there is not enough data available to sustain their qualification as single traits, including male sterility and glabrous skin. This short list and the doubts on some traits clearly indicate the paucity of studies on the genetical transmission of even the qualitative traits, which are generally easier to study than the quantitative ones.

Kernel taste

The first reference to the heritability of kernel taste was by Heppner (1923) who suggested a 3 : 1 distribution of sweet and bitter seedlings in the progeny of a large number of crosses, thus concluding that sweet kernel was dominant over bitter kernel and that most of the parents involved in the crosses were heterozygous for this trait. He was also the first to suggest that, if the original almond was bitter, a mutation occurred in this bitter almond with the sweet almond as a result. The mutant trait was thus dominant over the wild type and consisted in the loss of the bitter principle present in the wild progenitor.

Heppner (1926) confirmed these conclusions with a larger number of seedlings, as did all further studies (Dicenta and García 1993a; El Gharbi 1981; Grasselly 1972; Kester et al. 1977a, Vargas and Romero 1988). Only Spiegel-Roy and Kochba (1974) suggested that three genes could be involved in the determination of kernel taste, but they later discarded this three-gene hypothesis and accepted monofactorial determination (Spiegel-Roy and Kochba 1977, 1981).

The bitter taste in almond, as in the other stone fruits, is due to the production of the glucoside amygdalin. The immediate amygdalin precursor (prunasine) is not produced in the seed, but it is translocated from the mother plant to the developing seed. Thus, the pollen parent, which together with the seed parent determines the seed genotype, does not affect the taste of the seed. All the kernels of a tree will have either sweet or bitter kernels, and it is the mother plant that has the sweet or bitter genotype (Frehner et al. 1990). Only Crane and Lawrence (1952) have mentioned a case of xenia in almond taste, but their results have not been confirmed by any further research (Kumar and Das 1996). All of the research on almond taste shows that all of the fruits of a tree reflect the genotype of this tree.

The importance of kernel taste is not only due to the possible commercial acceptance of bitter kernels, which are used, in some products, including cakes and drinks, where a light bitter taste is especially appreciated, but also to the possible toxicity of the bitter component. The glucoside amygdalin, in the presence of water and the enzyme emulsin, both present in the kernel, is hydrolysed to benzaldehyde, hydrocyanic acid and glucose. It is the hydrocyanic acid that is toxic and bitter (McCarty et al. 1952).

Shell hardness

Shell hardness is related in almond to kernel percentage, and it is an important trait because of the different industrial processing of hard- or soft-shelled cultivars. In the Mediterranean regions hard-shelled cultivars are generally preferred because of their general better adaptation to non-irrigated culture, resistance to birds and some pests and better storing ability because of their slower rate of becoming rancid. However, in California and the new regions of almond culture, soft shelled cultivars are preferred.

Grasselly (1972) studied the crosses of a few cultivars and suggested that shell hardness was determined by a single gene with hard shell dominant over soft shell, thereby establishing the genotype of the parents involved in these crosses. However, this hypothesis has not been confirmed by other researchers who have considered shell hardness as a quantitative trait. This will be considered later.

Self-incompatibility alleles

Almond possesses a single locus gametophytic type of self-incompatibility (Socias i Company 1990). Although self-incompatibility was assessed in almond as early as 1919 (Tufts 1919), the identification of cross-incompatible groups and self-incompatibility alleles has been slow and it is still relatively unknown. This type of work can only be done in a group of related cultivars, and it has only been advanced with some Californian cultivars (Kester et al. 1994a). Cases of cross-incompatibility are not frequent (Socias i Company 1990) and are only found among cultivars deriving from the same

population or the same breeding programme. This situation could be the case of two Portuguese cultivars, 'Côco Grado' and 'Côco Miúdo' (Almeida 1949), belonging to the same population, and of two French cultivars, 'Ferragnès' and 'Ferralise' (Crossa-Raynaud and Grasselly 1985), very closely related genetically since they were developed from the same breeding programme.

Long-term observations on Californian cultivars led to the establishment of tentative cross-incompatibility groups (Kester and Asay 1975). Subsequent data on controlled pollinations confirmed these groups, which have been supplemented, with newly developed cultivars (Kester et al. 1994a). However, the work carried out in California was essentially independent from that initiated in France (Crossa-Raynaud and Grasselly 1985), with only a single cultivar in common and with a different identification system of the self-incompatibility alleles (letters in California and numbers in France). A common terminology has been recently adopted (Kester and Gradziel 1996), thus allowing better characterization both of the *S* alleles and of the cross-incompatibility groups, which now number up to 13.

The identification of these alleles has been by pollination studies, requiring long and tedious work. Recently, the development of stylar ribonuclease zymograms correlated with incompatibility alleles (Bošković and Tobutt 1996) has made this technique applicable to almond, where some of the previously identified alleles have been assigned to specific zymograms and new cases of cross-incompatibility have been detected (Duval et al. 1996). These results provide the possibility of advancing the assimilation of the alleles of the Californian and European groups of cultivars.

A mutation of the *S* allele could also be a trait qualitatively inherited. Kester et al. (1994b) have described a mutation conferring unilateral incompatibility in 'Nonpareil' because of the production of a nil allele, showing that the *S* locus can undergo different types of mutations. Further research would be needed to ascertain which type of mutation has taken place, as it could be due to a non-sense mutation or to a deletion (Socias i Company 1995) because pollen from the mutant type does not appear to function on its styles.

Self-compatibility

Self-compatibility was discovered in almond in 1945 by Almeida, but no attention was paid to the issue until the 1970s. The establishment of its genetic basis is relatively recent and has been based on studies conducted concurrently with breeding programmes, involving a small number of seedlings in the offspring (Socias i Company 1990). After assessing the transmission of self-compatibility Socias i Company and Felipe (1977) suggested that self-compatibility was dominant over self-incompatibility and that the self-compatible cultivars used in the breeding programmes were heterozygous (Socias i Company 1984). The results of most breeding programmes (Grasselly et al. 1981; Grasselly and Olivier 1984; Jraidi and Nefzi 1988; Socias i Company and Felipe 1988) have confirmed this conclusion of dominance and heterozygosity of self-compatibility.

In some crosses deviations have been observed from the expected ratios of 1 : 1 (self-compatible × self-incompatible) or 3 : 1 (self-compatible × self-compatible). These deviations were explained by the presence of a common allele between the self-compatible pollen parent (S_fS_1) and the self-incompatible seed parent (S_1S_2); only the pollen grains carrying the S_f allele would be able to grow through the pistil of the seed parent and achieve fertilization, thereby giving rise to an offspring of only self-compatible seedlings (Dicenta and García 1993b; Grasselly et al. 1985). However, as this does not seem to be the case in all crosses where identical self-incompatibility alleles are involved (Socias i Company and Felipe 1994a), inbreeding or the presence of lethal or deleterious genes have been suggested to explain these deviations (Socias i Company 1990).

Self-compatibility in almond has been suggested to be allelic to the *S* locus of self-incompatibility alleles, although no results have confirmed this assumption (Socias i Company 1990). However, our results on the transmission of self-compatibility through several backcrosses to self-incompatible cultivars may be evidence that, as in *Lycopersicon peruvianum*, the mutation involving self-compatibility may have taken place at the *S* locus (Rivers and Bernatzky 1994).

Blooming time

Blooming time is a very important trait in almond because of all the fruit species it has traditionally shown the earliest blooming time. This early bloom restricted almond growing to regions with a low risk of spring frosts. However, over centuries of almond growing its culture has been expanded into inland regions where the occurrence of spring frosts plays an important role. Thus late blooming has become an important trait in almond cultivars and most almond breeding programmes are trying to develop later blooming cultivars in order to avoid frost damage, when temperatures are also higher and more favourable for pollination and fertilization (Kester and Asay 1975).

Blooming time is considered to be inherited quantitatively in most fruit species (Anderson and Seeley 1993) and most results confirm this type of transmission in almond, as will be seen later. However, Kester (1965b) suggested that in some progenies of the late-blooming budsport 'Tardy Nonpareil', a single dominant gene could be involved in determining the blooming date, since a bimodal distribution of blooming dates was

observed among the seedlings obtained. Similar results were also obtained with the same cultivar by Grasselly (1978).

The utilization of a selection derived from 'Tardy Nonpareil' has allowed the transmission of this late-blooming allele to be followed for several generations in order to see if its behaviour is the same for the different offsprings (Socias i Company et al. 1996a). In the case of crosses of two sibs, a 3:1 distribution also confirmed the dominance of this late blooming mutation over normal blooming time (Grasselly and Olivier 1985). Thus, blooming time in almond seems to be determined by a major gene (*Lb*), with late bloom dominant over early bloom, and by modifier genes inherited quantitatively (Socias i Company et al. 1996a, b).

Male sterility

A cultivar is determined to be male sterile by the production of tetrads within the pollen sacs but without pollen differentiation (M. Herrero unpublished; Vargas García and Romero Romero 1978). There have been no reports on the possible transmission of this trait, but it can be hypothesized that it is a monofactorial recessive trait, as is the case with male sterility in peach (Hesse 1975) and apricot (*Prunus armeniaca* L.) (Burgos and Ledbetter 1994).

Glabrous skin

The almond fruit is pubescent, but a form has been identified with a glabrous skin (Socias i Company 1993). No studies have been conducted on the transmission of this trait, but the origin of this clone and the similarity of its skin to that of the glabrous skin in peach, leading to the nectarine trait (Hesse 1975), may suggest that it is a monofactorial recessive trait. This clone was identified in an orchard in Morocco, where seed propagation is still common (Janick 1989). This orchard originated from seeds of another orchard where all of the trees were sibs, coming from a single tree. Thus, this mutation could have been in the original tree, being subsequently manifested in an F_2 population (C. Grasselly unpublished).

This example also shows that a detailed examination of unusual crosses could lead to the identification of many other traits in almond, as the amount of breeding and number of crosses of either related or unrelated cultivars are relatively low. Two more traits can be considered in this context – flesh colour and flower colour – because both have been observed in crosses among related parents (C. Grasselly unpublished). Yellow colour has been observed in the flesh and could be recessive to green in a similar way that yellow flesh colour is recessive to white in peach (Connors 1920). Pink flower colour could also be recessive to white, although several gradations in the expression of pink flower colour have been observed in different almond cultivars (A. J. Felipe, unpublished), behaving similarly as in peach (Lammerts 1945).

Quantitative traits

As compared to other fruit species, no detailed studies on the transmission of quantitative traits have been undertaken in almond. The information available mostly refers to the heritability of different traits. As the calculations have been made using crosses from breeding programmes, which themselves are not too numerous and, moreover, were carried out using a reduced number of parents, these heritabilities are sometimes different, depending on the breeding programme. Thus, the values obtained can only be considered as tentative, although they are informative about the behaviour of the different traits when advancing from one generation to the next one.

The traits studied so far are the ones considered to be the most important from the agronomical point of view and refer mostly to the phenological stages and to fruit and kernel traits due to their marketing importance. From this point of view it is necessary to consider that quality is a changing concept with time and that some traits considered to be very important for commercial quality now may not be considered as such in the future. The most important phenological traits are blooming time, duration and intensity of bloom, as well as the ripening season. The most intensively studied of the fruit traits are nut and kernel weight, kernel percentage, number of blank nuts, percentage of double kernels, and some kernel quality aspects as skin color, rugosity, ...

Blooming time

As mentioned before, blooming time is considered to be inherited quantitatively in most fruit species (Anderson and Seeley 1993). In almond, blooming date may change from year to year depending on the winter weather conditions. Although the blooming sequence of different cultivars is relatively constant over the years, small variations in the order of blooming may occur (Felipe 1977) due to differences in the chilling requirements (Tabuenca 1972) and heat requirements before bloom (Tabuenca et al. 1972). Thus, blooming scales have been developed in order to classify the almond cultivars independently of the year (Gülcan 1985), and scales have been applied when studying the heritability of almond blooming (Kester et al. 1973). However, the presence of very late blooming seedlings in some progenies (Grasselly and Olivier

1985; Socias i Company et al. 1996a) has made this scale rate of blooming unapplicable, and it was decided to change it at the tenth GREMPA (Group de Recherches et d'Études Mediterranéen pour l'Amandier) Colloquium in Meknès (Morocco) in October 1996.

Most of the results on the transmission of blooming time in almond show that this trait is inherited quantitatively (Grasselly 1972; Grasselly and Gall 1967; Kester 1965b; Vargas and Romero 1988). However, in only a few cases has this heritability been estimated. The first approach was that of Kester et al. (1973), who established a heritability of 0.804. The same authors (Kester et al. 1977b) later confirmed the value of this heritability, which was based on observations of 13 different parents, 20 families and 490 offspring. Dicenta et al. (1993a) used a similar number of parents but a larger number of families and offspring and decreased this value to 0.67, probably due to the wider genetic basis of the parents involved in the later study. In fact, cultivars from different geographical regions may possess different quantitative loci related to blooming time, since the use of late-blooming cultivars from different regions has probably allowed the accumulation of different quantitative genes, thus retarding the blooming time in almond much more than in other fruit species (Socias i Company et al. 1996a).

Although blooming time is considered at full bloom, several different measures can be taken to record blooming time, estimating different percentages of open flowers and defining first, full and final blooming times (Dicenta et al. 1993a; Socias i Company et al. 1996a). Dicenta et al. (1993a) estimated the values for these different phenological stages: first (0.73), full (0.78) and final (0.67) blooming time, although there is a high correlation among these stages which cannot be considered independent.

Leafing time is highly correlated with blooming time, although there are differences among the almond cultivars with respect to the time of leafing in relation to the time of blooming, with a bloom-leaf index variable or even negative (Buyukyilmaz and Kester 1976). However, the heritability of leafing times was found to be even higher than blooming time (0.829 as compared to 0.804) for the same families (Kester et al. 1977b).

Blooming duration

Differences in blooming duration are associated with the climatic conditions, mainly temperature, at the time of bloom (Bernad and Socias i Company 1995; Dicenta et al. 1993a). Its importance is only related to the climatic conditions during bloom, as a long bloom can avoid the negative effects of a frost at the beginning of bloom or bad weather conditions disturbing the bee pollination work. Dicenta et al. (1993a) found a very high year × family interaction for blooming duration due to this temperature effect, but did not rule out a small genetic interaction, although the heritability of this trait (0.20) was considered somewhat uncertain.

Blooming intensity

Blooming intensity is considered a primary requirement for the good productivity of an almond clone. Bloom density was considered to be a trait transmissible to the offspring (Grasselly 1972), thus opening the possibility of selection for this character, although no heritability of this trait was estimated until Dicenta et al. (1993a) established a value of 0.54.

Grasselly (1972) related bloom intensity to production precocity, with a possible correlation of the juvenile period of the seedlings and the unproductive period of the young orchard. Obviously, the juvenility of the seedlings does not allow the evaluation of this trait to occur until the forth or fifth year (Kester and Asay 1975).

Ripening season

The date of ripening is also highly affected by the year, but as for blooming time, the ripening sequence of different cultivars is highly constant. The importance of an early ripening season is due to the necessity of harvesting before the fall rains and, consequently, of offering the new crop to industry before the Christmas marketing orders, both of which are very important factors in some countries. Early harvest is also very important under non-irrigated conditions, before drought is too severe. Both Grasselly (1972) and Kester and Asay (1975) observed that ripening season was quantitatively inherited. While heritability has been estimated as 0.69 (Dicenta et al. 1993a) the presence of non-additive variance has been suggested.

Dicenta et al. (1993a) also considered the duration of maturity, with a heritability of 0.61. Although this trait was not previously considered in almond, a simultaneous ripening, thus a short ripening season, would be interesting for facilitating harvesting.

Production intensity

Production intensity is highly correlated with bloom intensity, but it also depends on the conditions of fruit setting. A medium bloom density with a high set can reach a higher production than a high bloom density with a low set (Bernad and Socias i Company 1997). Thus, although production intensity was considered to be quantitatively inherited (Grasselly 1972; Kester and Asay 1975), when its heritability was estimated, it was lower than that of bloom intensity (0.45 versus 0.54) (Dicenta et al. 1993a).

Fruit weight

Fruit weight in almond is considered to be the in-shell weight, including the kernel and the endocarp, but not the fleshy mesocarp which is normally eliminated at harvest. This parameter is not too important because it fluctuates according to the kernel percentage or the shell hardness of each cultivar and because the commercial part of the fruit is the kernel.

Fruit weight is variable from year to year, mostly depending on the production characteristics of the season, mainly the crop level, although in almond the crop effect on the fruit size is less important than in other fruit trees (A.J. Felipe unpublished). However, in spite of this variation, the heritability of this trait is high, being established by Kester et al. (1977a) at 0.81. Estimations by other researchers (Dicenta et al. 1993b; Spiegel-Roy and Kochba 1981) agreed with that value.

Kernel weight and shape

Kernel weight is also variable from year to year, even more than fruit weight. As the kernel is the commercial part of the almond nut, its weight and shape are very important, as different sizes and shapes are required for different industrial applications (Berger 1969). Kernel weight heritability was first estimated by Kester et al. (1977a) at 0.64, and later estimations agreed with this value (Dicenta et al. 1993b; Spiegel-Roy and Kochba 1981).

Kernel shape may vary according to the different cultivars, since it is maintained as a cultivar trait (Gülcan 1985). The linear dimensions of the kernel, length, width and thickness, have been defined as commercial characteristics in almond as well as the length/width ratio (Kester 1965b; Kester et al. 1980). Grasselly (1972) observed the transmission of these linear dimensions, but their heritabilities were not estimated until Kester et al. (1977a): length, 0.77; width, 0.62; thickness, 0.71.

Shell hardness

In spite of the suggestion by Grasselly (1972) that shell hardness is quantitatively inherited, it seems that the regression analysis of the kernel percentage does not reflect the presence of dominance (Dicenta et al. 1993b). However, the different parents used in the estimation of the heritability of this trait may have created a bias on the evaluation of its transmission and the estimation of its heritability. Thus, Kester et al. (1977a) estimated this heritability at 0.55 whereas Spiegel-Roy and Kochba (1981) increased this value to 0.82. Dicenta et al. (1993b) agreed with the former with a value of 0.56.

Double kernels

The presence of double kernels in almond is due to the fertilization of the two ovules in the almond ovary. This is considered to be a negative trait, lowering fruit quality rating depending on their proportion (Kester et al. 1980). This is due to the fact that when two kernels are produced in the same fruit, they are deformed and make the commercial processes of cracking, size selection and peeling difficult. The percentage of double kernels is a cultivar trait but presents large variations depending on the sample and the year. While some physiological and climatic reasons have been pointed out as possible causes of these variations, none has been clearly defined. Particularly low temperatures before blooming (Egea and Burgos 1994) or at blooming time (Grasselly and Gall 1967; Rikhter 1969; Spiegel-Roy and Kochba 1974) have been mentioned as promoting higher percentages of double kernels. The earliest blooming flowers seem to be the ones that produce the largest number of double kernels (Socias i Company and Felipe 1994b). Palasciano et al. (1993) reported that an optimized pollination also increases the percentage of double kernels.

Although this is a complex trait needing additional investigation for its elucidation (Asensio and Socias i Company 1996) it seems that the ability to produce double kernels is a quantitative trait that can be partially inherited; its expression, however, can be modified by different causes (Socias i Company and Felipe 1994b). Grasselly (1972) first suggested a quantitative component on the transmission of this trait, and Spiegel-Roy and Kochba (1974) showed that this transmission was complicated by environmental influences making its heritability fairly unpredictable. Kester et al. (1977a) estimated this heritability at 0.51, but with a very high standard deviation. Similar low estimates were also reported by Vargas and Romero (1988) and Dicenta et al. (1993b). However, the utilization of parents with no double kernels or with a low percentage of them in the different crosses, as this is considered a negative trait, may have distorted the estimation of its heritability.

Other fruit characters

Several other fruit characters have been studied, but not so intensively, probably because their economic importance is much lower than that of fruit weight or the percentage of double kernels. Thus Kester et al. (1977a) considered that there was no transmission for hull dehiscence, the extent of hull opening at harvest (0.02), and for shell colour (0.05). Kernel colour showed a higher heritability (0.42) but was highly inconsistent, showing strong environmental effects and also being dependent in part on the stage of maturity at harvest as well as on the length and type of storage. Low

heritabilities were also obtained for sealed shell (0.42) and width of opening (0.21) with large yearly fluctuations in the cultivars subject to this type of default, including the main Californian cultivar 'Nonpareil' (Kester and Asay 1975).

Several kernel traits are conditioned largely by the genotype but with some fluctuations in different years. Penetrance may be involved since only some kernels are affected and only in some years. These traits include crease, a deep depression in the side of some kernels (0.79), shrivelling (0.36), pubescence (0.30) and grade (0.28). These traits are mostly rated subjectively, so inconsistencies in measurement may be a factor limiting the significance of their heritability (Kester et al. 1977a). Worm damage shows a large genotype-year interaction, indicating that damage is greater to certain genotypes whenever the pests are found, with a heritability of 0.30. However, shell hardness is a resistance component to worm damage, as hard shells offer a barrier to the entrance of the worm inside the shell.

Disease and frost resistance

Although almond has been considered to be a species resistant to some pests and diseases as well as to abiotic factors, not much attention has been paid to evaluate specific resistances and to estimate their heritability. Only Grasselly (1972) reported the transmission of resistance to fungal diseases to the offspring of 'Ardéchoise', but he did not evaluate its transmission rate. Grasselly (1981) also reported that there is a general resistance or suceptibility to different fungal diseases in the same cultivars, thus implicating some correlation among these traits. El Gharbi (1981) reported the transmission of *Taphrina deformans* (Berk.) Tul. susceptibility from 'Tuono' to its offspring. Similarly, Felipe (1988) has suggested that frost resistance could also be a quantitative factor to be transmitted to the offspring as observed in 'Tuono' and its progenies.

Other morphological and physiological characters

A quantitative transmission has been suggested for several other traits in almond, but difficulty in their measurement or the low level of quantitative observations in a reduced number of offspring have not allowed any estimation of their heritability. Among these traits, Grasselly (1972) pointed out that growth habit is a complex trait, with an apparent dominance in some offspring probably due to the parents used in the crosses, but quantitatively inherited. Kester and Asay (1975) also reported that the different morphological types of tree structure are evidently polygenic in nature, transmitted to the offspring and highly heritable.

The colour and linear dimensions of the leaf are also traits with a possible quantitative transmission, although they are not of great agronomical interest (Grasselly 1972). This fact possibly explains similarities for leaf traits among seedlings coming from the same cross (Bernad and Socias i Company 1994). These same conclusions can also be applied to flower dimensions (Bernad and Socias i Company 1994; Grasselly 1972) and to stamen number (Grasselly 1972).

Almond is a species with a very difficult propagation by hardwood cuttings. However, Felipe (1984) identified an almond cultivar with a very good rate of hardwood propagation and has shown that this ability can be transmitted to the offspring (Felipe 1992). Although this transmission has not been quantified, it is another example of a quantitative trait.

Molecular markers

Any morphological trait can be considered as a marker of its own expression, but the availability of markers has considerably increased recently with the development of molecular genetics. In fruit trees, morphological markers are not frequent, and only a few cases have been reported, such as the gland type and powdery mildew resistance in peach (Connors 1921). In almond, the low level of genetically identified traits makes the utilization of morphological markers impossible. Thus, only molecular markers can be considered.

There is a wide range of different approaches to developing molecular markers useful in genetical studies (Staub et al. 1996). Most of these have been studied, or are being studied at the present time in almond, as in other fruit tree species. This is in contrast to the relatively limited knowledge gained in the field of classical genetics.

In the strictest sense, molecular markers can be considered to be qualitative traits because of their Mendelian transmission. They are therefore also useful in identifying cultivars, tracing genealogies and estimating possible similarities among different cultivars.

Enzymes

Isoenzymes were the first molecular markers used in the identification of almond cultivars, their usefulness being shown by their environmental stability, their codominant expression and the reproducibility of results. The first application was the identification of cultivars (Hauagge et al. 1987a; Cerezo et al. 1989), the identity being confirmed, in different plant tissues (Cerezo and Socias i Company 1992). Transmission of the different isoenzymes in a Mendelian fashion was verified by Hauagge et al. (1987b), a genetical validation of their study, which was also applied to the confirmation of parentages (Bernad and Socias i Company 1994) and the rate of pollen migration under commercial conditions (Jackson and Clarke 1991).

The variability observed in almond was larger than that observed in peach (Arulsekar et al. 1986), probably due to the higher heterozygosity of such an outcrossing species as almond, which has also been subject to less breeding efforts than peach. For this reason the variability observed in the Californian cultivars (Hauagge et al. 1987a), where more breeding has been carried out, is smaller than that observed in the Zaragoza collection (Cerezo et al. 1989) which includes representatives of a wider range of geographical regions (Socias i Company and Felipe 1992b).

The utility of enzymes as molecular markers is reduced, as in other species, by the paucity of isozyme loci, a problem increased by the low variation in some loci. Hauagge et al. (1987a) studied 7 enzyme systems but 3 of them did not show variation. Cerezo et al. (1989) studied 9 enzyme systems, with variation in all of them. Arús et al. (1994b) increased this number to 10, making a total amount of 15 enzyme systems. All the studies involving enzyme heritability and linkage analysis include a reduced number of enzyme loci, 4 (Asíns et al. 1994) or 7 (Vezvaei et al. 1995; Viruel et al. 1995), thus allowing further approaches with the remaining loci.

Restriction fragment length polymorphisms (RFLPs)

RFLPs provide the means of developing a very large number of molecular markers and, thus, of not only detecting linkage among markers but also of constructing genetic maps. The first report in almond (Viruel et al. 1995) studied 120 RFLPs in the F_1 progeny of a cross between the two cultivars 'Ferragnès' and 'Tuono'. The first genetic map in almond was thus constructed, which also included 7 isozyme loci. Research is under way to increase the number of RFLP loci in almond (Arús et al. 1996; de Vicente et al. 1996) and to obtain more detailed maps. It is interesting to consider that some of the probes used to develop markers in this study correspond to known genes in almond (Garcia-Mas et al. 1992, 1995; Stöcker et al. 1993) or in peach (Lee et al. 1990).

Furthermore, RFLPs are found to be homologous across wide phylogenetic ranges and are adequate for genome comparisons. Thus, a study in almond is homologous to one in other stone fruit species (*Prunus* genus), and the results are comparable (Arús et al. 1994a, 1996).

Random amplified polymorphic DNAs (RAPDs)

RAPDs have also been studied in almond, leading to the identification of 56 RAPDs (Joobeur 1996) in the same population as that studied by Viruel et al. (1995), allowing their localization in the same map, thereby increasing its density and length. With these markers, as well as with RFLPs, research is under way to increase the number of loci (Arús et al. 1996; de Vicente et al. 1996).

Linkage studies

Prior to the availability of molecular markers there had been different approaches to studying the correlation among different morphological and physiological traits. The correlation among some of these traits is evident, as for the first, full, and final flowering times (Dicenta and García 1992), because these are sequential events that did not evolve independently, and the correlation coefficients estimated (ranging from 0.87 to 0.96) only reflect the constancy of the blooming sequences through the years (Felipe 1977).

The negative correlation between flowering time and flowering duration only reflects the fact that the later the flowering time the higher the temperature at bloom, thus making the blooming season shorter, as usually observed (Bernad and Socias i Company 1995). The negative correlation between flowering time and flowering density and production was observed mainly in the offspring of 'Tardy Nonpareil' (Grasselly 1978; Kester 1965a) and is thus only applicable to some progenies and not to the species as a whole. This same rationality may apply to the correlation between flowering time and maturity time, considered to be slightly positive (Dicenta and García 1992), although different observations show that late blooming cultivars may show early ripening as well (Kumar et al. 1993; Socias i Company and Felipe 1992b).

Another evident correlation is flowering density and productivity (Dicenta and García 1992) because only with especially unfavourable pollination conditions may this close relationship be broken. Also evident is the correlation between fruit and kernel weight, especially if the shell hardness is similar. The correlation between the percentage of double kernels and kernel percentage (Casella 1970) may be explained because fruits with double kernels normally leave less empty space inside the almond shell.

All these correlations do not show real linkages between two characters because either their correlation coefficients are too low or the correlation is only evident because of the parallel variation of the two traits. In some cases the correlations only apply to a specific cultivar and its offspring.

The first linkage reported in almond was that of two linkage groups of isozyme loci, one of four genes, *PGM-2–GPI-2–AAT-2–LAP-2*, and the other of two genes, *IDH-2–AAT-1* (Arús et al. 1992). These linkages were partially confirmed by Vezvaei et al. (1995), who identified two linkage groups of two enzyme loci each, one with *AAT-1 - IDH*, and the other with *LAP-1–PGM-2*, suggesting also the possible linkage of *LAP-1–GPI-2*. There appears to be some discrepancy with the results

of Arús et al. (1992), but the difference could be due to a different numbering of the enzyme loci by the different authors. Thus, both results can be considered in agreement.

Later a linkage was reported between RFLPs and enzyme loci (Viruel et al. 1995), which allowed the development of the first map in this species, as they identified eight linkage groups, corresponding to the haploid number of almond (Darlington 1930). As mentioned before, the saturation of this map has been increased with RAPDs (Joobeur 1996) and newly identified molecular markers (Arús et al. 1996; de Vicente et al. 1996).

These linkages, however, are limited at the moment to molecular markers, and they will only be useful in breeding if there is linkage with agronomically interesting traits. The first attempt to establish this type of linkage was that of Asíns et al. (1994), with 4 enzyme systems and 16 quantitative traits. However, the strong genetic × environment interaction detected showed that possible correlations observed in some years were not maintained in other years, thus questioning the value of this approach. Even if we accept that 17 putative quantitative trait loci (QTLs) were detected, only 3 of them have behaved homogeneously over the years, suggesting a differential gene expression depending on the year, due to the genotype × environment interaction. The temperature effect suggested in some cases may be difficult to ascertain because all the seedlings were affected by the same temperatures and the same seedlings were subjected to the same records through the years of this study. As the stability of the possible quantitative effects detected is essential for perennial plant improvement, this approach could not lead to the establishment of any real linkage between a molecular marker and a trait in almond.

Another attempt was that of Joobeur (1996) using bulk segregation analysis to target the kernel bitterness and the self-compatibility loci in two different progenies. No marker was found for self-compatibility but two markers were found flanking the bitterness locus, at distances of 22 ± 9 and 18 ± 9 cM. These distances, however, are probably too large for practical use of these markers in a selection process. The identification or manipulation of alleles at desired target loci with single markers requires tight linkages, probably less than 5 cM (Stuber 1992).

Further attempts have been undertaken (Arús et al. 1996; de Vicente et al. 1996), but no results are yet available. Thus, so far, no valid linkages have been described in almond.

Concluding remarks

This review of almond genetics shows the paucity of knowledge available from a classical genetics position, whereas the mapping of molecular markers has advanced at a similar pace as in other fruit trees. Almond, as apple (Weeden 1996), has a high level of heterozygosity, which makes genetic studies not only feasible but also surprisingly easy to perform. There is an additional point of interest since the similarity between almond and peach at the genetic level suggests a high level of homology at the molecular marker level between these two species and even throughout the *Prunus* genus (Arús et al. 1994a). Thus, some of the mapping work in peach has been done with some peach × almond hybrid progenies (Arús et al. 1994a; Warburton et al. 1996), illustrating the possibility of obtaining new markers and trying to apply the markers developed in peach to traits not yet well defined in almond, as already mentioned for glabrous skin and male sterility.

There may be some difficulties in applying molecular biology techniques because of the particular constitution of fruit trees. In order to tag any gene of interest with a selection fidelity of 99%, it would be necessary to have marker loci spaced at 20 cM intervals throughout the genome (Tanksley 1983). This level of spacing would be very difficult to attain in any fruit species. Besides, although the homologous regions already mentioned are found in different species, mapping is different even for cultivars of the same species (Viruel et al. 1995), thus making it difficult to establish definite linkages in the regions where there is no homology. And, even if some parents have been repeatedly used in different breeding programmes (Kester and Gradziel 1996) and some have been recommended for their combining ability (García et al. 1994), this is not often the case in practical breeding because in any successive cross to create a new offspring the best outcome of the previous crosses will be used instead of the original parents. The utilization of different parents in each cross might require the creation of new maps for the specific variable regions in any new parent involved in the crosses.

An alternative approach useful in almond and other fruit and nut tree species may be the use of such techniques as bulked segregant analysis (Michelmore et al. 1991) where no previous knowledge of the genome or development of a genetic map is required to obtain markers linked to a particular gene of interest.

The actual situation, however, is one of genetic maps quite well-defined for genetic markers, but with no localization of agronomical traits; this is especially true in almond. This can be due to the low level of efforts dedicated to the classical study of fruit trees, involving a large amount of space and long-term observations, mainly in the orchard, work not excessively attractive to some researchers. As pointed out by Socias i Company (1997) it is easier to utilize the trees as a source of plant material to be analysed in the laboratory than to follow the trees' behaviour in the orchard. Differing appreciations of these two types of study may lead to a disequilibrium in the information available, possibly

making the genetic markers useless unless supported by the appropriate and well known plant material. Both molecular technologies and field based research are vital to breeding programmes (Scorza 1996), and any improvement in plant material will come only if both are fully coupled. The shortcoming that has resulted from this uncoupling is that, to date, no cultivar developed from marker-assisted selection has been publicly released (Staub et al. 1996), although some advanced selections are nearly available for some grains and vegetable crops, but not for any fruit species.

Another difficulty may arise from the fact that different traits may be linked to different types of markers, thus making necessary the analysis of the offspring for these different markers and increasing considerably the cost of this evaluation. The ideal objective would be to have all the agronomical interesting traits linked to the same type of molecular markers so that only a single anlaysis would be necessary to select the interesting traits.

The ultimate objective of plant breeding is to offer new cultivars that will enable the grower to make profits from an orchard managed properly (Socias i Company et al. 1997). This aim includes the production of a good crop of high-quality fruit. Both of these concepts, quality and quantity, are sometimes difficult to be defined. Quality especially is an idea evolving with time which in almond has had to be defined within a framework of specific commercial uses (Berger 1969). On the other hand, the classical components of agricultural yield, dimensions, weight and number, generally exhibit continuous variation, and differences in their expression are usually governed by multiple gene systems (Gottlieb 1984). However, the study of quantitative traits is quite difficult because a distinct linkage between the quantitative trait and the marker is not recognized unless the quantitative trait has a considerable effect and the linkage is tight (Lavi et al. 1994). The number of progeny required for this analysis could be quite large, at the level of hundreds or even thousands, numbers not easily attained with fruit trees.

It is not unexpected that metric characters have been found to be influenced by five to ten or more genes (Lande 1981). However, when estimates of genes controlling yield are made, it is not strange that all of the genes of a plant affect yield (Wallace et al. 1972), and although this is clearly a maximum estimate, it is closer to reality than a minimum estimate. The realization that yield components are influenced by diverse physiological and morphological processes may induce the quantitative geneticist to try to assess all of the loci in a population that contribute to the genetic variance, even though many of them do so only as a consequence of their general effects on growth and vigour (Gottlieb 1984). For characters with low heritabilities the application of molecular markers will be only possible when additive genetic variation is associated with the marker (Staub et al. 1996).

All these facts indicate that probably more attention has to be paid to the elucidation of more qualitative traits and to those quantitative traits in which a major effect would be due to a major quantitative trait locus. The effectiveness of any marker assisted selection procedure will depend on the accuracy of the phenotypic classification of the expression of any trait and the degree of linkage between a marker and the trait of interest (Staub et al. 1996). For the breeder, a molecular map is not a goal but a tool to reach a goal: the improved cultivar (Scorza 1996). This implies more field work and the recognition of this work because any new cultivar has to be grown in an orchard and has to allow the grower to earn his living.

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